

Towards the Plant Golgi Proteome

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The plant cell wall is comprised of complex sugar polymers including cellulose, hemicellulose and pectin. The Golgi apparatus within the plant cell produces a large proportion of these polysaccharides prior to their incorporation into the cell wall. The details of how these complex sugars are synthesized and delivered are currently poorly understood. Our current knowledge of the protein constituent that comprise the plant Golgi is relatively poor when compared to other subcellular components within the cell. In order to further understand the role of this organelle in cell wall biosynthesis we are characterizing this subcellular compartment using proteomics. We are employing an orthogonal approach which utilizes density centrifugation followed by charge based separation of the organelle on a Free Flow Electrophoresis system. Analysis of Golgi purified fractions from Arabidopsis cell culture by mass spectrometry after FFE separation indicates the method is suitable for isolation of this organelle from plants. We have identified 300 to 400 proteins from these fractions and found approximately 50 glycosyl transferases likely involved in matrix polysaccharide biosynthesis. Overall approximately 50% of the proteins in this list are of known or likely Golgi in origin; approximately 35% are unknown or are derived from the endosomal system and 15% appear to be contaminants from other organelles and membranes. Fluorescent protein constructs are being designed to confirm localizations of novel and ambiguous proteins. This technique will enable us to commence in-depth comparative cell wall proteomics focusing on protein function and changes and in the Golgi apparatus.

This work was part of the DOE Joint BioEnergy Institute (<http://www.jbei.org>) supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy.